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Photosynthate translocation increases in response to low seawater pH in a coral–dinoflagellate symbiosis

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Abstract. This study has examined the effect of low seawater pH values (induced by an increased CO₂ partial pressure) on the rates of photosynthesis, as well as on the carbon budget and carbon translocation in the scleractinian coral species *Stylophora pistillata*, using a new model based on ¹³C labelling of the photosynthetic products. Symbiont photosynthesis contributes to a large part of the carbon acquisition in tropical coral species, and it is thus important to know how environmental changes affect this carbon acquisition and allocation. For this purpose, nubbins of *S. pistillata* were maintained for six months at two pH_Ts (8.1 and 7.2, by bubbling seawater with CO₂). The lowest pH value was used to tackle how seawater pH impacts the carbon budget of a scleractinian coral. Rates of photosynthesis and respiration of the symbiotic association and of isolated symbionts were assessed at each pH. The fate of ¹³C photosynthates was then followed in the symbionts and the coral host for 48 h. Nubbins maintained at pH_T 7.2 presented a lower areal symbiont concentration, and lower areal rates of gross photosynthesis and carbon incorporation compared to nubbins maintained at pH_T 8.1. The total carbon acquisition was thus lower under low pH. However, the total percentage of carbon translocated to the host as well as the amount of carbon translocated per symbiont cell were significantly higher under pH_T 7.2 than under pH_T 8.1 (70 % at pH_T 7.2 vs. 60 %

at pH_T 8.1), such that the total amount of photosynthetic carbon received by the coral host was equivalent under both pHs (5.5 to 6.1 μg C cm⁻² h⁻¹). Although the carbon budget of the host was unchanged, symbionts acquired less carbon for their own needs (0.6 compared to 1.8 μg C cm⁻² h⁻¹), explaining the overall decrease in symbiont concentration at low pH. In the long term, such decrease in symbiont concentration might severely affect the carbon budget of the symbiotic association.

1 Introduction

Changes in seawater chemistry that are induced by an increase in partial-pressure CO₂ (*p*CO₂) include a decrease in pH, in the carbonate ion (CO₃²⁻) concentration and in the saturation states of calcium carbonate minerals (reviewed in Hoegh-Guldberg et al., 2007; Doney et al., 2009). These changes are deleterious for many marine organisms, both for photosynthetic and calcifying species such as tropical scleractinian corals (Kroeker et al., 2010). They may induce a decrease in the pH at the calcification site of corals (Venn et al., 2013) and a decrease in the calcification rates (reviewed in Erez et al., 2011). Experiments performed at very low pHs (< 7.5) to constrain coral physiological performances

have shown not only a complete decalcification in some coral species but also an increase in tissue biomass and general survival under these extreme conditions (Fine and Tchernov, 2007). Contrary to calcification, the effect of small or large changes in $p\text{CO}_2/\text{pH}$ on the physiology of the coral symbionts has been much less studied. However, symbionts, through photosynthesis, are the major actors in the nutrient acquisition of the symbiotic association (Muscattine et al., 1981, 1984; Grover et al., 2002), which in turn is a key process in corals and in animal in general that determines their growth, fecundity and survival.

Coral symbionts, called *Symbiodinium*, belong to the eukaryotic algal taxon of dinoflagellates. This taxon had form II RuBisCO (ribulose-1,5 biphosphate carboxylase/oxygenase), which has a low CO_2 affinity compared to the other algal taxa (reviewed in Tortell, 2000). Corals have therefore developed carbon-concentrating mechanisms (CCM; Leggat et al., 1999), but it has been suggested that the symbionts in hospite are still limited by the host in dissolved inorganic carbon (DIC) because they are dependent on host bicarbonate (HCO_3^-) uptake (Goiran et al., 1996; Marubini and Thake, 1999; Marubini et al., 2008). In theory, an increase in seawater CO_2 concentration, leading to a parallel increase in bicarbonate concentration, should increase the rates of symbiont photosynthesis. However, the few studies performed on the subject led to controversial results, differing according to the coral species studied or to the acclimation length to the seawater chemistry changes. Experiments performed on a relatively short-term scale found no effect of acidification (pH ranging from 7.5 to 7.9) on areal rates of coral photosynthesis (Scheinder and Erez, 2006; Marubini et al., 2008; Godinot et al., 2011; Houlbrèque et al., 2012) or in the maximum photosynthetic capacity per cell (P_{max} , Crawley et al., 2010). Longer term experiments at low pH values (7.4 to 7.8), however, measured a decrease in these rates or in the photosynthetic efficiency of the symbionts (Reynaud et al., 2003; Anthony et al., 2008; Iguchi et al., 2012; Kaniewska et al., 2012), often coupled with bleaching (i.e. the loss of symbionts; Anthony et al., 2008). Only the temperate species *Cladocora caespitosa* did not significantly change its photosynthesis after being maintained for one year under pH 7.8 (Rodolfo-Metalpa et al., 2010). This different inter-specific response to low pH might be related both to the extent and/or length of pH decrease and to the symbiont phylotype, as demonstrated in a recent study (Brading et al., 2011), in which the cell-specific productivity of one phylotype out of four was increased under low pH, while it remained unchanged for the others.

In terms of carbon acquisition for the coral host, not only rates of photosynthesis but also photosynthate translocation rates are important. It is usually assumed that autotrophic carbon acquisition by the host is directly related to the rates of symbiont photosynthesis because symbionts are supposed to translocate more than 90 % of the photosynthates to the host for its own nutrition (Muscattine et al., 1981). However,

percentage of translocation may vary according to the host species (Davies, 1984, 1991; Edmunds and Davies, 1986), the symbiont genotype (Loram et al., 2007) or the environment (Muscattine et al., 1984; Tremblay et al., 2012a), and is therefore independent of the production level.

In this study, we measured the fate of the photosynthetically acquired carbon, as well as the amount of carbon translocated from the symbionts to the host, in colonies of the scleractinian coral *Stylophora pistillata* (Esper, 1797) maintained under two pH_T s (8.1 and 7.2) for six months. For this purpose, a new model of carbon translocation (Tremblay et al., 2012b), based on the isotopically labelled ^{13}C bicarbonate, was used. Although the low pH value chosen in this study is lower than that expected under future climate scenarios, it is useful to examine the physiological response of corals to environmental hypercapnia (Barry et al., 2010). It also helps constrain their boundaries of performance (e.g. Kurihara and Shirayama, 2004) in order to guide subsequent studies on similar taxa. The objectives of our study were thus to determine the effect of decreased pH on the autotrophic carbon acquisition by the dinoflagellates in symbiosis with *S. pistillata*, and to relate symbiont productivity to carbon acquisition by the coral host. In other words, considering that acidification decreases symbiont productivity in some scleractinian species, do we observe a parallel decrease in carbon acquisition by the host, or does the host maintain its carbon acquisition by increased percentage of carbon translocation?

2 Materials and methods

2.1 Biological material

Three colonies of the scleractinian coral *S. pistillata* (Pocilloporidae) were sampled at 10 m depth in the Gulf of Eilat (Israel). A total of 54 nubbins (18 nubbins per colony) were prepared by cutting the apical branches of the colonies. They were then equally divided (i.e. nine nubbins from each colony) in two tanks (150 L), which were maintained at two pH_T s: 8.1 (or $\text{pH}_{\text{NBS}} = 8.2$ and $p\text{CO}_2 = 387 \mu\text{atm}$, ambient conditions) and 7.2 (or $\text{pH}_{\text{NBS}} = 7.3$ and $p\text{CO}_2 = 3898 \mu\text{atm}$, Table 1) for six months. For this purpose, seawater was continuously pumped at 30 m depth into 1000 L tanks, equipped with a pH electrode (S-200C, Sensorex, CA, USA) connected to a pH controller (Aquastar, IKS Computer System GmbH, Karlsbad, Germany). The controller continuously monitored pH and adjusted it to the desired value by bubbling CO_2 (from a CO_2 cylinder). Daily pH variability was low (± 0.05) throughout the experiment, and no major fluctuations were recorded. Well-mixed filtered (500 μm) water from each tank continuously flowed into smaller tanks containing the coral nubbins. All pH data were recorded using monitoring software (Timo, Matuta, Germany) on the NBS scale. The pH_{NBS} data were shifted onto the total pH_T scale by subtracting 0.1, which includes a minor correction

Table 1. Carbonate chemistry parameters of the treatment and the control calculated from pH, total alkalinity (TA), temperature (25 °C) and salinity (40) using the program CO2SYS (Lewis and Wallace, 1998). DIC: dissolved inorganic carbon; Ω_{arag} : aragonite saturation state.

| pH total scale (pH _T) | TA ($\mu\text{eq kg}^{-1}$) | DIC ($\mu\text{mol kg}^{-1}$) | $p\text{CO}_2$ (μatm) | $\text{CO}_2(\text{aq})$ ($\mu\text{mol kg}^{-1}$) | HCO_3^- ($\mu\text{mol kg}^{-1}$) | CO_3^{2-} ($\mu\text{mol kg}^{-1}$) | Ω_{arag} |
|--------------------------------------|----------------------------------|------------------------------------|---------------------------------------|---|---|---|------------------------|
| 8.1 | 2501 | 2122 | 387 | 10.6 | 1846 | 265 | 4.02 |
| 7.2 | 2501 | 2544 | 3898 | 107.1 | 2393 | 44 | 0.67 |

for $[\text{SO}_4^{2-}]$ and the stability constant of HSO_4^- at a salinity of 40.7 (Krief et al., 2010). Nubbins were fed once a week with *Artemia salina* nauplii and mashed fish mix. The tanks were kept under an irradiance of $\sim 140 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (10 h light: 14 h dark photoperiod) provided by metal halide lamps (400 W/D, Osram GmbH, Germany) in an open-flow system (renewal rate of two times the volume of the basin per day) at $\sim 25^\circ\text{C}$. The in situ light level was approximately equal to $280 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at noon. Temperature was regulated using a combination of an array of 300 W BluClima aquarium heaters (Ferplast Spa, Vicenza, Italy) and a water cooler (custom-made at Interuniversity Institute for Marine Science). From previous studies (Krief et al., 2010), we know that *S. pistillata* grows well under both pH_Ts for years, and the difference between 7.2 and 8.1 allows us to clearly establish if there is a pH effect on the autotrophic carbon budget of *S. pistillata*. Moreover, the dominant clade of *Symbiodinium* hosted by colonies of *S. pistillata* used in this study was determined to the clade level according to Santos et al. (2002) through use of chloroplastic ribosomal deoxyribonucleic acid sequences. In colonies sampled in situ, as well as in nubbins maintained at the two pHs, clade A was dominant and did not change during the experiment.

2.2 Rates of photosynthesis and respiration

Rates of respiration (R) and net photosynthesis (P_n) were measured in each experimental condition using three nubbins (one nubbin per colony). Measurements were performed using temperature-controlled chambers coupled with optodes (ProODO, YSI, Yellow Springs, OH, USA) and filled with 580 mL of 0.65 μm -filtered seawater at the right pH. Optodes were calibrated before each measurement using sodium bisulfite (NaHSO_3) and water-saturated air as 0 and 100 % oxygen saturation values, respectively. Seawater temperature in the chambers was maintained at $25.0 \pm 0.5^\circ\text{C}$, and was continuously stirred using magnetic stirrers. For each colony, photosynthesis at $140 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and post-illumination dark respiration were measured over one hour. Rates of gross photosynthesis (P_g) were calculated by adding R to P_n . Samples were then frozen for later determinations of symbionts, chlorophyll (Chl) and protein concentrations. For this purpose, nubbins were thawed and their tissue was detached from the skeleton using an air

brush and 0.2 μm -filtered seawater. The slurry was homogenized using an electric homogenizer (Heidolph DIAX 100 with a generator 6G, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Subsamples were taken for the determination of the symbiont concentration according to Rodolfo-Metalpa et al. (2006), and protein concentration according to Smith et al. (1985) using the BC Assay Protein Quantitation Kit (Uptima, Interchim) and a Xenius[®] spectrofluorometer (Safas, Monaco). The remaining sample was used to assess Chl a and Chl c_2 concentrations according to Tremblay et al. (2012c) by use of a spectrophotometer UVmc²[®] (Safas, Monaco). Data were subsequently normalized to the skeletal surface area of each nubbin ($\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$) measured using the wax-dipping technique (Veal et al., 2010) or per symbiont cell ($\mu\text{mol O}_2 \text{cell}^{-1} \text{h}^{-1}$).

In addition to the above measurements, respiration rates of freshly isolated symbionts were also determined from three nubbins per condition (one per colony). Symbionts were therefore extracted in 0.65 μm -filtered seawater at the right pH using an air brush, homogenized and centrifuged at 3000 g for 5 min. The pellet, containing the symbionts was resuspended in 0.65 μm -filtered seawater at the right pH. Respiration rates and symbiont concentration were measured as described above.

The autotrophic carbon acquired (P_C) and respired (R_C) was calculated for each treatment, by converting oxygen fluxes to carbon equivalents using the molar weights, as $P_C = P_g \times 12/PQ$ and $R_C = R \times 12 \times RQ$ (Anthony and Fabricius, 2000), where PQ and RQ are photosynthetic and respiratory quotients equal to 1.1 mol O_2 :mol C and 0.8 mol C:mol O_2 , respectively, for *S. pistillata* of the Red Sea (Muscatine et al., 1981; Gattuso and Jaubert, 1990).

2.3 H^{13}CO_3 labelling experiments

The experiments were performed according to Tremblay et al. (2012b). Briefly, corals were placed in $\text{H}^{13}\text{CO}_3^-$ ($\text{NaH}^{13}\text{CO}_3$ 98 atom % ^{13}C , #372382, Sigma-Aldrich, St-Louis, MO, USA) enriched seawater and then transferred into non-enriched seawater for various chase periods. At the end, samples were frozen for later ^{13}C enrichment measurements in the symbionts and coral tissue.

For each condition, 15 beakers were therefore filled with 200 mL of seawater at the right pH, enriched with a concentration of 0.6 mM $\text{NaH}^{13}\text{CO}_3$ (or 23 % ^{13}C enrichment of

Table 2. List of symbols, definition and units.

| Symbol | Definition |
|---------------------|---|
| C | Carbon |
| C_{inc} | ^{13}C enrichment of the incubation medium (%) |
| C_{L} | Amount of C lost ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$ or %) |
| C_{meas} | ^{13}C measured in the sample (%) |
| C_{nat} | Natural abundance in ^{13}C in control nubbins (%) |
| C_{R} | Percentage of fixed C remaining in symbionts and host tissue (%) |
| M_{C} | Mass of C per milligram of tissue or symbionts ($\mu\text{g mg}^{-1}$) |
| M_{sample} | Mass of the freeze-dried sample (mg) |
| P_{C} | Gross C fixed photosynthetically by symbionts ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$) |
| P_{g} | Oxygen produced by gross photosynthesis ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) |
| P_{n} | Oxygen produced by net photosynthesis ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) |
| PQ | Photosynthetic quotient (equal to 1.1 mol O_2 :mol C) |
| R | Oxygen consumed by respiration of holobiont ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) |
| R_{C} | C respired by holobiont ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$) |
| R_{H} | C respired by coral host ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$) |
| RQ | Respiratory quotient (equal to 0.8 mol C:mol O_2) |
| R_{S} | C respired by symbionts ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$) |
| S | Nubbin surface area (cm^2) |
| T_{S} | Amount of C translocated; calculated from the symbiont rates ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$ or %) |
| t_{chase} | Incubation time of the nubbins in the non-enriched incubation medium in the light (h) |
| t_{pulse} | Incubation time of the nubbins in the enriched incubation medium (h) |
| ρ_{DOC} | C incorporation rate in released DOC (not measured) |
| ρ_{H} | C incorporation rate in coral tissue ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$) |
| ρ_{POC} | C incorporation rate in released POC (not measured) |
| ρ_{S} | C incorporation rate in symbiont ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$) |

the incubation medium) and maintained at the same irradiance and temperature as described previously. Fifteen nubbins (five per colony) were individually incubated over 5 h in the ^{13}C -enriched seawater and thereafter transferred into 15 other beakers containing non-enriched seawater at the right pH (chase). Three nubbins (one per colony) were removed after 0, 2, 4, 24 and 48 h and immediately frozen at -20°C . Six control nubbins per condition (two per colony; incubated from the beginning in 200 mL non-enriched seawater) were run in parallel and two were sampled after 0, 24 and 48 h and immediately frozen at -20°C . The pH_{NBS} was controlled after addition of the ^{13}C bicarbonate and during the whole experiment to ensure that it remained at the desired level, especially for the low pH condition.

All nubbins were treated according to Tremblay et al. (2012b). Briefly, tissue was detached from the skeleton using an air brush in $0.2\mu\text{m}$ -filtered seawater. The slurry was homogenized using an electric homogenizer (Heidolph DIAX 100 with a generator 6G), and the animal and symbionts fractions separated by centrifugation. Samples were frozen and freeze-dried until analysis. The % ^{13}C and the carbon content of the animal tissue and symbionts were determined with a mass spectrometer (Delta Plus, Thermofisher Scientific, Bremen, Germany) coupled with a C/N analyser (Flash EA, Thermofisher Scientific).

2.4 Autotrophic carbon budget calculations

The equations used to calculate autotrophic carbon budget are fully described in Tremblay et al. (2012b). However, in the present study, ρ_{POC} was not measured and the equations were adjusted accordingly and listed below. The carbon incorporation rate (ρ) in the symbionts (ρ_{S}) and animal tissue (ρ_{H}), expressed in $\mu\text{g C cm}^{-2} \text{ h}^{-1}$ was calculated as follows:

$$\rho = \frac{(C_{\text{meas}} - C_{\text{nat}}) \times M_{\text{sample}} \times M_{\text{C}}}{(C_{\text{inc}} - C_{\text{meas}}) \times (t_{\text{pulse}} + t_{\text{chase}}) \times S}, \quad (1)$$

where C_{meas} and C_{nat} are the percentages of ^{13}C measured in enriched and control samples, respectively, C_{inc} is the percent ^{13}C enrichment of the seawater (which varies during the chase; see Tremblay et al., 2012b), M_{sample} is the mass of the sample (mg), M_{C} is the carbon content per symbiont or host tissue biomass ($\mu\text{g mg}^{-1}$), S is the surface area (cm^2), t_{pulse} and t_{chase} are the incubation time (h) in the enriched incubation medium and non-enriched medium in the light, respectively. See Table 2 for a list of symbols and their definitions.

The percentage of fixed carbon remaining (C_{R}) in symbionts and host tissue is calculated by dividing ρ_{S} or ρ_{H} by the gross photosynthesis expressed in carbon (P_{C}), and then

multiplying by 100:

$$C_R = (\rho_S \text{ or } \rho_H / P_C) \times 100. \quad (2)$$

The carbon acquired through photosynthesis (P_C) may have different fates: it is either respired by the coral assemblage (R_C), incorporated into the symbiont (ρ_S) and host (ρ_H) biomass or lost in the surrounding water as POC (ρ_{POC}) and DOC (ρ_{DOC}). Therefore, the carbon budget equation is

$$P_C = R_C + \rho_S + \rho_H + \rho_{POC} + \rho_{DOC}. \quad (3)$$

R_C includes two components, i.e. symbiont (R_S) and host (R_H) respiration; $R_S + R_H = R_C$.

The above equation does not consider the carbon incorporated in the skeleton because it has been shown that 25 to 30 % comes directly from the external medium as dissolved inorganic carbon, and 70 to 75 % from internal respiration R_C (Erez, 1978; Furla et al., 2000); the first component is external to the equation, and the second component is part of R_C . It follows from Eq. (3) that the amount of carbon lost as combined R_C , ρ_{DOC} and ρ_{POC} (C_L) is

$$C_L = R_C + \rho_{DOC} + \rho_{POC} = P_C - \rho_S - \rho_H. \quad (4)$$

The amount of carbon translocated by symbionts to the host (T_S) corresponds to the total amount of carbon gained by photosynthesis (P_C) minus the sum of the carbon retained in symbionts (ρ_S) and respired by them (R_S):

$$T_S = P_C - \rho_S - R_S. \quad (5)$$

The percentage of carbon lost (C_L) and translocated (T_S) was obtained by dividing C_L or T_S by P_C and multiplying by 100.

In the present study, ρ_{DOC} and ρ_{POC} were not measured but estimated from the carbon lost equation (Eq. 4):

$$\rho_{DOC} + \rho_{POC} = C_L - R_C = (P_C - \rho_S - \rho_H) - R_C. \quad (6)$$

2.5 Statistical analysis

All parameters were expressed as average value \pm standard error of the mean (s.e.m.). Data were checked for normality using a Kolmogorov–Smirnov test with Lilliefors correction and for variance homoscedasticity using a Levene test. When the conditions were not fulfilled, data were transformed (natural logarithm for protein content). Significant differences in physiological parameters were tested using a t test. The effect of pH on the incorporation rates (ρ), the percentage of fixed carbon remaining (C_R), the carbon lost (C_L) and carbon translocation (T_S) was tested using a repeated measures analysis of variance (ANOVA). Colonies were considered as “subjects”, while pH was the between subject factor. The second factor was time over the course of the chase interval, and had five levels (0, 2, 4, 24 and 48 h). The repeated measures ANOVA was followed by a posteriori test (Tukey test) if significant. Differences in the amount of carbon lost (C_L) and

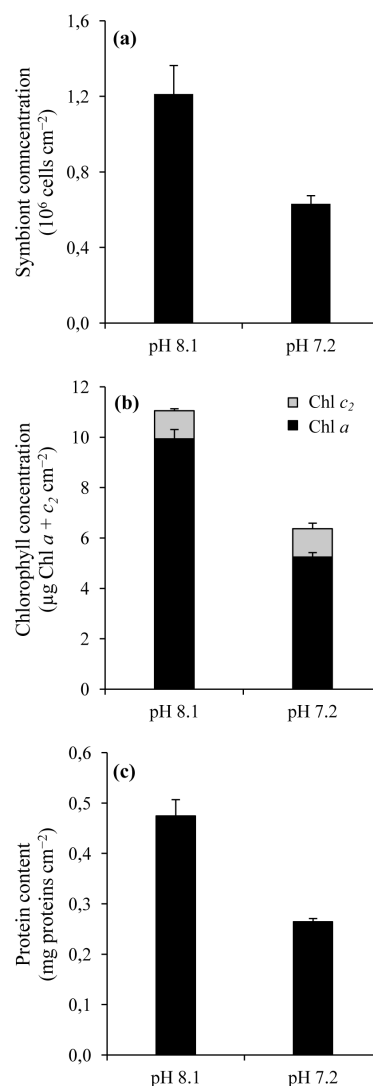


Fig. 1. (a) Symbiont, (b) chlorophyll and (c) protein concentrations for *Stylophora pistillata* at pH_T 8.1 and 7.2. Data are means \pm standard error of means of $n = 4$ measurements.

total respiration rates of the nubbins (R_C) were tested using a t test. Differences between factors were considered significant for a p value < 0.05 . Statistics were performed using Systat 13 (Systat Software, Chicago, IL, USA).

3 Results

3.1 Effect of seawater pH on the main physiological parameters

Carbonate chemistry parameters in the different conditions are presented in Table 1. Seawater acidification induced significant differences in several physiological parameters of *S. pistillata* (Figs. 1 and 2). Areal symbiont concentration was 48 % lower at pH_T 7.2, compared to pH_T 8.1 (Fig. 1a,

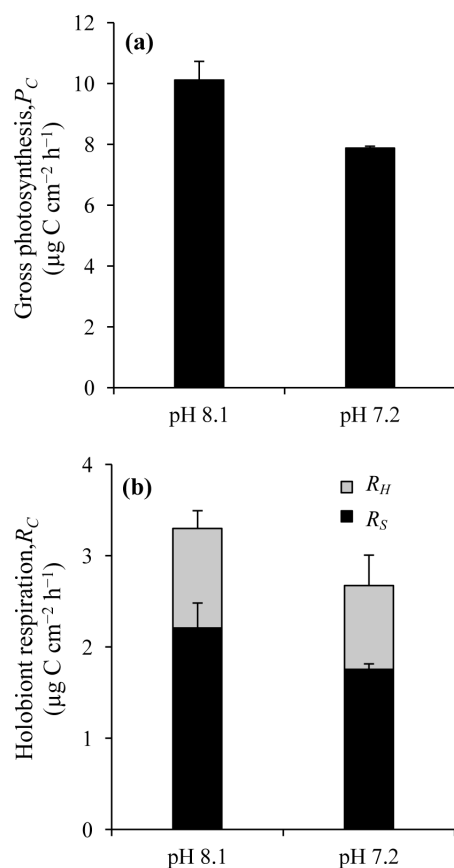


Fig. 2. (a) Gross photosynthesis (P_C) and (b) holobiont respiration ($R_C = R_S + R_H$) for *Stylophora pistillata* at pH_T 8.1 and 7.2. Data are means \pm standard error of means of $n = 3$ measurements.

t test $p = 0.0045$; $t = 4.42$; $df = 6$), combined with a 42 % decrease in areal Chl a concentration at pH_T 7.2, compared to pH_T 8.1 (Fig. 1b, t test $p < 0.0001$; $t = 12.85$; $df = 6$). On the contrary, there was no difference in areal Chl c_2 concentration between the two treatments (Fig. 1b, t test $p = 0.8097$; $t = 0.25$; $df = 6$). Protein content was significantly lower at pH_T 7.2, compared to pH_T 8.1 (Fig. 1c, t test $p = 0.0010$; $t = 6.83$; $df = 5$).

As a result of low symbiont and chlorophyll concentrations in corals maintained under pH_T 7.2, areal rates of gross photosynthesis were 22 % lower at this pH compared to those measured at pH_T 8.1 (Fig. 2a, t test $p = 0.0153$; $t = 4.06$; $df = 4$), whereas the respiration rates were similar (Fig. 2b, t test $p = 0.1286$; $t = 1.91$; $df = 4$), corresponding to ca. $72 \mu\text{g C cm}^{-2} \text{d}^{-1}$ in both conditions. Gross photosynthesis therefore supplied 22 % less carbon to nubbins maintained under pH_T 7.2 ($79 \pm 1 \mu\text{g C cm}^{-2} \text{d}^{-1}$) compared to those maintained under pH_T 8.1 ($101 \pm 6 \mu\text{g C cm}^{-2} \text{d}^{-1}$). When photosynthetic rates were normalized per symbiont cell (cell-specific productivity), they were slightly but significantly higher (t test $p = 0.0347$; $t = 3.15$; $df = 4$) un-

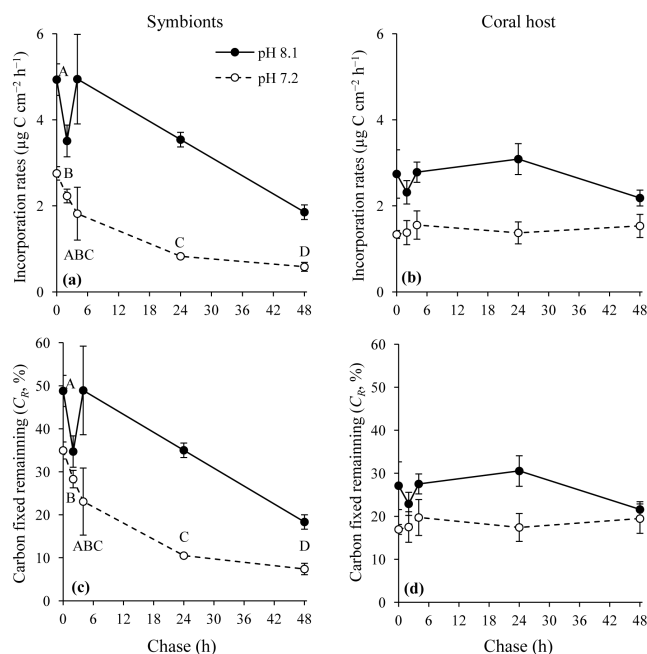


Fig. 3. Carbon incorporation rates (ρ) in (a) symbionts and (b) coral host. Percentage of fixed carbon that remains (C_R) in the (c) symbionts and (d) host for *Stylophora pistillata* at pH_T 8.1 and 7.2. Data are means \pm standard error of means of $n = 3$ measurements. Chase intervals with different letters (A to D) are significantly different.

der pH_T 7.2 ($11.8 \pm 0.5 \times 10^{-6} \mu\text{g C cell}^{-1} \text{h}^{-1}$) than under pH_T 8.1 ($9.4 \pm 0.6 \times 10^{-6} \mu\text{g C cell}^{-1} \text{h}^{-1}$; not shown in a figure).

3.2 Carbon translocation between the symbionts and their host

The natural percentage of ^{13}C (measured in non-enriched control samples) was lower at pH_T 7.2 (1.1224 ± 0.0002 and 1.1222 ± 0.0001 atom % ^{13}C for symbiont and coral tissue, respectively) than at pH_T 8.1 (1.1294 ± 0.0014 and 1.1306 ± 0.0004 atom % ^{13}C , respectively). After incubation in ^{13}C bicarbonate, nubbins from both conditions were however enriched in ^{13}C compared to control samples (atom % ^{13}C ranged between 1.6296 and 3.0147 % in symbionts, and between 1.3015 and 1.3877 % in the host tissue).

Carbon incorporation rates at the beginning of chase interval (0h) in the symbionts and host tissue were twice lower under pH_T 7.2 (2.8 and $1.3 \mu\text{g C cm}^{-2} \text{h}^{-1}$, respectively) than under pH_T 8.1 (4.9 and $2.7 \mu\text{g C cm}^{-2} \text{h}^{-1}$, respectively; Fig. 3a, b and Table 3). The amount of carbon retained in symbionts decreased during the chase to reach 0.6 and $1.8 \mu\text{g C cm}^{-2} \text{h}^{-1}$ in nubbins maintained at pH_T 7.2 and 8.1, respectively (Fig. 3a and Table 3). As a consequence, only 8 to 18 % remained in this fraction after 48h (Fig. 3c and Table 3). In contrast, carbon retained in the coral tissue remained more or less constant during the whole chase (Fig. 3b

Table 3. Results of the repeated measures analysis of variance (ANOVA) testing the effect of pH and incubation time on the carbon incorporation rates (ρ) and percentage of fixed carbon (C_R) in the symbionts and coral host. Significant p values are in bold.

| Factor | Degrees of freedom | Symbionts p | F value | Coral host p | F value |
|--|--------------------|------------------|-----------|-------------------|-----------|
| <i>Incorporation rate (ρ)</i> | | | | | |
| Between subjects | | | | | |
| pH | 1 | 0.0005 | 103.30 | 0.0060 | 28.36 |
| Error | 4 | – | – | – | – |
| Within subjects | | | | | |
| Time | 4 | 0.0002 | 10.44 | 0.5992 | 0.71 |
| Time \times pH | 4 | 0.1968 | 1.71 | 0.4432 | 0.99 |
| Error | 16 | – | – | – | – |
| <i>Fixed carbon remaining (C_R)</i> | | | | | |
| Between subjects | | | | | |
| pH | 1 | 0.0026 | 44.59 | 0.0454 | 8.25 |
| Error | 4 | – | – | – | – |
| Within subjects | | | | | |
| Time | 4 | 0.0001 | 11.66 | 0.6536 | 0.62 |
| Time \times pH | 4 | 0.2141 | 1.64 | 0.4651 | 0.94 |
| Error | 16 | – | – | – | – |

and Table 3) and corresponded to 19–22 % of the initial fixed carbon (Fig. 3d and Table 3).

The major part of the fixed carbon was lost as respiration and excretion of DOC and/or POC. Total losses accounted for 3.8 and 2.4 $\mu\text{g C cm}^{-2} \text{h}^{-1}$ at the beginning of the chase for corals under pH_T 7.2 and 8.1, respectively, and reached 6.1 and 5.8 $\mu\text{g C cm}^{-2} \text{h}^{-1}$ after 48 h (Fig. 4a and Table 4), corresponding to 60 to 73 % of the fixed carbon (Fig. 4b and Table 4). The amount of carbon lost after 48 h (Fig. 4a), at both pHs, was significantly higher than the respiration rates measured using the respirometry technique (Fig. 2b, t test $p = 0.0025$; $t = 6.76$; $\text{d.f.} = 4$ for pH_T 7.2 and t test $p = 0.0020$; $t = 7.22$; $\text{d.f.} = 4$ for pH_T 8.1). These results suggest that the release of autotrophic carbon as DOC and POC must have accounted for a significant fraction of the carbon lost. Indeed, the difference between the C_L and R_C values after 48 h was equal to 3.1 and 2.8 $\mu\text{g C cm}^{-2} \text{h}^{-1}$ or 39 and 27 % of the fixed carbon for nubbins under pH_T 7.2 and 8.1, respectively.

Carbon translocation rates are represented in Fig. 4c and d. The total amount of carbon translocated per skeletal surface area was similar under the two pHs (Fig. 4c and Table 4), despite higher areal rates of photosynthesis under pH_T 8.1 (Fig. 2a), and increased with time (Fig. 4c and Table 4). Therefore, the percentage of the total fixed carbon translocated was significantly higher at pH_T 7.2 than at pH_T 8.1 (Fig. 4d and Table 4). Indeed, 70 % of the fixed carbon was translocated to the host after 48 h at pH_T 7.2 compared to 60 % at pH_T 8.1 (Fig. 4d). Moreover, due to lower areal symbiont concentration at pH_T 7.2 compared to pH_T 8.1, the amount of carbon translocated per symbiont cell

was 40 % higher after 48 h in symbionts of nubbins maintained at pH_T 7.2 ($8.8 \pm 0.2 \times 10^{-6} \mu\text{g C cell}^{-1} \text{h}^{-1}$) than in symbionts of corals under pH_T 8.1 ($5.0 \pm 0.1 \times 10^{-6} \mu\text{g C cell}^{-1} \text{h}^{-1}$; not shown in a figure).

4 Discussion

Exposure of *S. pistillata* to pH_T of 7.2 led to a reduction in areal symbiont concentration and subsequently in the areal rates of gross photosynthesis compared to control corals maintained at pH_T 8.1. However, this decreased capacity in autotrophic carbon acquisition under low pH was counterbalanced by a higher percentage of carbon translocation, leading to an equivalent amount of autotrophic carbon acquired by the host in both pH conditions.

As previously and recently observed for one clade of *Symbiodinium*, clade A2 (Brading et al., 2011), cell-specific productivity in the present study was increased under low pH – i.e. under high seawater CO_2 and bicarbonate concentrations. The higher cell-specific productivity observed under pH_T 7.2 compared to pH_T 8.1 could be due to the direct effect of increased CO_2 /bicarbonate in seawater or to the indirect “bleaching effect”, which decreased areal symbiont concentration in the host tissue and therefore increased the availability of light and DIC for each symbiont cell. This suggests that in hospite symbionts of *S. pistillata* are DIC limited. Such limitation was already observed in two previous studies (Goiran et al., 1996; Marubini et al., 2008). Marubini et al. (2008) indeed measured an increase in areal rates of gross photosynthesis of *S. pistillata*

Table 4. Results of the repeated measures analysis of variance (ANOVA) testing the effect of pH and incubation time on the amount and percentage of carbon lost (C_L) and translocated (T_S). Significant p values are in bold.

| Factor | Degrees of freedom | Amount ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$) p | F value | Percentage (%) p | F value |
|--|--------------------|--|-----------|-----------------------|-----------|
| <i>Carbon lost (C_L)</i> | | | | | |
| Between subjects | | | | | |
| pH | 1 | 0.0461 | 8.15 | 0.0067 | 26.66 |
| Error | 4 | — | — | — | — |
| Within subjects | | | | | |
| Time | 4 | 0.0071 | 5.20 | 0.0052 | 5.60 |
| Time \times pH | 4 | 0.2513 | 1.49 | 0.2972 | 1.34 |
| Error | 16 | — | — | — | — |
| <i>Carbon translocation (T_S)</i> | | | | | |
| Between subjects | | | | | |
| pH | 1 | 0.1828 | 2.59 | 0.0029 | 42.03 |
| Error | 4 | — | — | — | — |
| Within subjects | | | | | |
| Time | 4 | 0.0002 | 10.44 | 0.0001 | 11.64 |
| Time \times pH | 4 | 0.1968 | 1.71 | 0.2162 | 1.63 |
| Error | 16 | — | — | — | — |

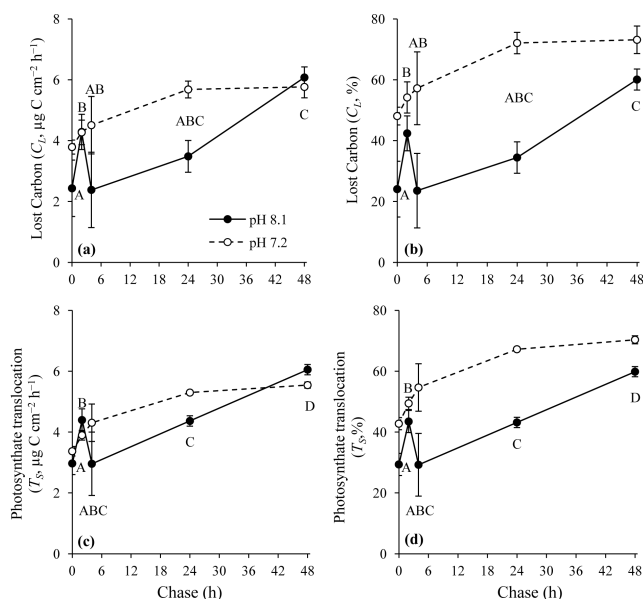


Fig. 4. (a) Amount and (b) percentage of carbon lost by symbiosis (C_L); (c) amount and (d) percentage of photosynthesized carbon translocated to the host by symbionts (T_S) for *Stylophora pistillata* at pH_T 8.1 and 7.2. Data are means \pm standard error of means of $n = 3$ measurements. Chase intervals with different letters (A to D) are significantly different.

when bicarbonate concentration was increased in seawater, independently of the pH conditions. In addition, Goiran et al. (1996) showed that the reduction in the rate of net photosynthesis per chlorophyll in the species *Galaxea fascicu-*

laris was higher in a system in which bicarbonate was decreased rather than in a system with increased bicarbonate and low pH. Altogether, these results suggest that DIC limitation of the photosynthesis is a general feature for several coral species and their associated symbionts, despite the presence of CCM. It has been shown that CCM efficiency differs between algal species (Tortell, 2000), and coral symbionts might have particularly low efficiencies.

Despite an enhancement of the cell-specific productivity, areal rates of gross photosynthesis were lowered at pH_T 7.2 because of the lowering of the zooxanthellae concentration in the corals subject to long-term seawater acidification. This decrease in symbiont concentration was also observed for a lower decrease in pH_T (Krief et al., 2010; Kaniewska et al., 2012). As a consequence, the total autotrophic carbon acquired by the symbiotic association, estimated from the areal rates of gross photosynthesis, was 22 % lower at pH_T 7.2 than at pH_T 8.1 ($7.9 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ instead of $10.1 \mu\text{g C cm}^{-2} \text{ h}^{-1}$). This decrease in autotrophic carbon acquisition, also observed in previous long-term expositions to relatively low pHs (Reynaud et al., 2003; Anthony et al., 2008; Iguchi et al., 2012; Kaniewska et al., 2012), can have detrimental impacts for the carbon budget and the health of the corals. The model used in this study (summarized in Fig. 5), however, shows that the coral host acquired the same amount of autotrophic carbon under both pHs (5.5 and $6.1 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ for pH_T 7.2 and 8.1, respectively). This was achieved following an increase in the amount of carbon translocated per symbiont cell, and in the percent of translocation in corals maintained at pH_T 7.2 compared to those at pH_T 8.1. Since respiration rates of the host and the symbionts

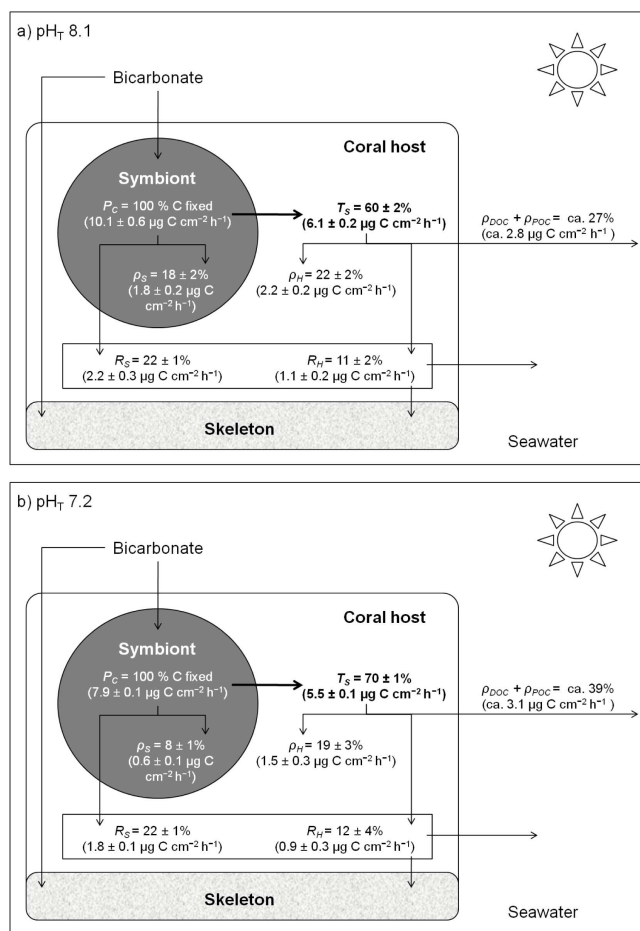


Fig. 5. Mass-balanced results of photosynthate translocation and carbon budget in *Stylophora pistillata* under (a) pH_T 8.1, and (b) pH_T 7.2 based on ¹³C experiments after 48 h. Symbols are defined in the text and summarized in Table 2. Data are means \pm standard error of means of $n = 3$ measurements.

remained unchanged under both conditions (ca. 1 and 2 $\mu\text{g C cm}^{-2} \text{ h}^{-1}$ or 12 and 22 % of the total carbon was respired by the host and the symbionts, respectively, under both pHs), as were the losses of carbon as DOC and POC (ca. 3 $\mu\text{g C cm}^{-2} \text{ h}^{-1}$ under both pHs), the host conserved in both conditions ca. 20 % of the autotrophically acquired carbon in its tissue after 48 h, although with a slightly lower total amount for corals under pH_T 7.2 than pH_T 8.1 (1.5 compared to 2.2 $\mu\text{g C cm}^{-2} \text{ h}^{-1}$). This lower amount matches with the lower amount of proteins.

The higher percent of translocation in corals at pH_T 7.2 can be the result of a lower carbon demand of the symbionts (in lower areal concentration in the host tissue), and/or to a higher demand from the host through, for example, an increased release of host factors (HRFs) (Muscatine, 1967; Grant et al., 1998; Davy and Cook, 2001). Overall, this observation is beneficial for the host over time, which can maintain a certain protein level (although lower at pH_T 7.2

than at pH_T 8.1), but not for the symbionts, which are the losers under low pH. Indeed, the amount of carbon remaining in the symbionts after 48 h dropped from 18 % (or 1.8 $\mu\text{g C cm}^{-2} \text{ h}^{-1}$) of the photosynthesized carbon in corals at pH_T 8.1 to 8 % (or 0.6 $\mu\text{g C cm}^{-2} \text{ h}^{-1}$) in corals at pH_T 7.2 (Fig. 5). In other words, there was a 33 % decrease in the symbiont cell-specific carbon incorporation rate at pH_T 7.2 compared to pH_T 8.1 (from $1.5 \times 10^{-6} \mu\text{g C cell}^{-1} \text{ h}^{-1}$ at pH_T 8.1 to $0.9 \times 10^{-6} \mu\text{g C cell}^{-1} \text{ h}^{-1}$ at pH_T 7.2). Such low carbon incorporation in symbiont cells at pH_T 7.2 is the best explanation for the bleaching observed. Symbionts have fewer building blocks to construct new biomass or to grow and replace the dying cells. This observation therefore mitigates the positive effect, on the host carbon budget, of higher percent carbon translocation. Under the very low pH_T conditions such as those applied in this study, the coral host can survive for some time with the same amount of autotrophically acquired carbon, but it is at the detriment of its population of symbionts. This population had severely declined after six months at pH_T 7.2 compared to the population of control corals, and might continue decreasing up to the level at which there will be no photosynthate production and translocation anymore. For the host, the increased photosynthate translocation cannot even maintain its biomass in the long term since the total protein content has decreased in colonies maintained at pH_T 7.2. Questions remaining open are whether the symbiotic association can remain on the fragile equilibrium observed in this study in the long term or if the symbiont physiology will further decline; or how the symbiotic association will be impacted when maintained very long term under a smaller pH/ $p\text{CO}_2$ perturbation; and, finally, what will be the cross effect of increased $p\text{CO}_2$ and seawater temperature on the carbon translocation and budget in such symbioses.

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References

- Anthony, K. R. N. and Fabricius, K. E.: Shifting roles of heterotrophy and autotrophy in coral energy budgets at variable turbidity, *J. Exp. Mar. Biol. Ecol.*, 252, 221–253, doi:10.1016/S0022-0981(00)00237-9, 2000.

- Anthony, K. R. N., Kline, D. I., Diaz-Pulido, G., Dove, S., and Hoegh-Guldberg, O.: Ocean acidification causes bleaching and productivity loss in coral reef builders, *Proc. Natl. Acad. Sci. USA*, 105, 17442–17446, doi:10.1073/pnas.0804478105, 2008.
- Barry, J. P., Tyrrell, T., Hansson, L., and Gattuso, J.-P.: CO₂ targets for ocean acidification perturbation experiments, in: Guide to best practices for ocean acidification research and data reporting, edited by: Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.-P., Publications Office of the European Union, Luxembourg, 53–66, 2010.
- Brading, P., Warner, M. E., Davey, P., Smith, D. J., Achterberg, E. P., and Suggetta, D. J.: Differential effects of ocean acidification on growth and photosynthesis among phylotypes of *Symbiodinium* (Dinophyceae), *Limnol. Oceanogr.*, 56, 927–938, doi:10.4319/lo.2011.56.3.0927, 2011.
- Crawley, A., Kline, D. I., Dunn, S., Anthony, K. R. N., and Dove, S.: The effect of ocean acidification on symbiont photorespiration and productivity in *Acropora formosa*, *Glob. Change Biol.*, 16, 851–863, doi:10.1111/j.1365-2486.2009.01943.x, 2010.
- Davies, P. S.: The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*, *Coral Reefs*, 2, 181–186, 1984.
- Davies, P. S.: Effects of daylight variations on the energy budgets of shallow-water corals, *Mar. Biol.*, 108, 137–144, 1991.
- Davy, S. K. and Cook, C. B.: The influence of “host release factor” on carbon release by zooxanthellae isolated from fed and starved *Aiptasia pallida* (Verrill), *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, 129, 487–494, 2001.
- Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A.: Ocean acidification: the other CO₂ problem, *Ann. Rev. Mar. Sci.*, 1, 169–192, doi:10.1146/annurev.marine.010908.163834, 2009.
- Edmunds, P. J. and Davies, P. S.: An energy budget for *Porites porites* (Scleractinia), *Mar. Biol.*, 92, 339–347, 1986.
- Erez, J.: Vital effect on stable-isotope composition seen in foraminifera and coral skeletons, *Nature*, 273, 199–202, 1978.
- Erez, J., Reynaud, S., Silverman, J., Scheinder, K., and Allemand, D.: Coral calcification under ocean acidification and global change, in: Coral reefs: an ecosystem in transition, edited by: Dubinsky, Z., and Stambler, N., Springer, Heidelberg, 151–176, 2011.
- Fine, M. and Tchernov, D.: Scleractinian coral species survive and recover from decalcification, *Science*, 315, 1811, doi:10.1126/science.1137094, 2007.
- Furla, P., Galgani, I., Durand, I., and Allemand, D.: Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis, *J. Exp. Biol.*, 203, 3445–3457, 2000.
- Gattuso, J.-P. and Jaubert, J.: Effect of light on oxygen and carbon dioxide fluxes and on metabolic quotients measured in situ in a zooxanthellate coral, *Limnol. Oceanogr.*, 35, 1796–1804, 1990.
- Godinot, C., Houlbrèque, F., Grover, R., and Ferrier-Pagès, C.: Coral uptake of inorganic phosphorus and nitrogen negatively affected by simultaneous changes in temperature and pH, *PLoS ONE*, 6, e25024, doi:10.1371/journal.pone.0025024, 2011.
- Goiran, C., Al-Moghrabi, S., Allemand, D., and Jaubert, J.: Inorganic carbon uptake for photosynthesis by the symbiotic coral /dinoflagellate association I. Photosynthetic performances of symbionts and dependence on sea water bicarbonate, *J. Exp. Mar. Biol. Ecol.*, 199, 207–225, 1996.
- Grant, A. J., Rémond, M., and Hinde, R.: Low molecular-weight factor from *Plesiastrea versipora* (Scleractinia) that modifies release and glycerol metabolism of isolated symbiotic algae, *Mar. Biol.*, 130, 553–557, 1998.
- Grover, R., Maguer, J. F., Reynaud-Vaganay, S., and Ferrier-Pagès, C.: Uptake of ammonium by the scleractinian coral *Stylophora pistillata*: Effect of feeding, light, and ammonium concentrations, *Limnol. Oceanogr.*, 47, 782–790, 2002.
- Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., Harvell, C. D., Sale, P. F., Edwards, A. J., Caldeira, K., Knowlton, N., Eakin, C. M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R. H., Dubi, A., and Hatzitolos, M. E.: Coral reefs under rapid climate change and ocean acidification, *Science*, 318, 1737–1742, doi:10.1126/science.1152509, 2007.
- Houlbrèque, F., Rodolfo-Metalpa, R., Jeffrey, R., Oberhänsli, F., Teyssier, J.-L., Boisson, F., Al-Trabeen, K., and Ferrier-Pagès, C.: Effects of increased pCO₂ on zinc uptake and calcification in the tropical coral *Stylophora pistillata*, *Coral Reefs*, 31, 101–109, doi:10.1007/s00338-011-0819-2, 2012.
- Iguchi, A., Ozaki, S., Nakamura, T., Inoue, M., Tanaka, Y., Suzuki, A., Kawahata, H., and Sakai, K.: Effects of acidified seawater on coral calcification and symbiotic algae on the massive coral *Porites australiensis*, *Mar. Environ. Res.*, 73, 32–36, doi:10.1016/j.marenvres.2011.10.008, 2012.
- Kaniewska, P., Campbell, P. R., Kline, D. I., Rodriguez-Lanetty, M., Miller, D. J., Dove, S., and Hoegh-Guldberg, O.: Major cellular and physiological impacts of ocean acidification on a reef building coral, *PLoS ONE*, 7, e34659, doi:10.1371/journal.pone.0034659, 2012.
- Krief, S., Hendy, E. J., Fine, M., Yam, R., Meibom, A., Foster, G. L., and Shemesh, A.: Physiological and isotopic responses of scleractinian corals to ocean acidification, *Geochimica Cosmochim. Acta*, 74, 4988–5001, doi:10.1016/j.gca.2010.05.023, 2010.
- Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G.: Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms, *Ecol. Lett.*, 13, 1419–1434, doi:10.1111/j.1461-0248.2010.01518.x, 2010.
- Kurihara, H. and Shirayama, Y.: Effects of increased atmospheric CO₂ on sea urchin early development, *Mar. Ecol. Prog. Ser.*, 274, 161–169, 2004.
- Leggat, W., Badger, M. R., and Yellowlees, D.: Evidence for an inorganic carbon-concentrating mechanism in the symbiotic dinoflagellate *Symbiodinium* sp., *Plant Physiol.*, 121, 1247–1255, 1999.
- Lewis, E. and Wallace, D. W. R.: Program developed for CO₂ System Calculations, ORNL/CDIAC-105, Oak Ridge Natl. Lab., US Dep. Energy, Oak Ridge, Tenn., 1998.
- Loram, J. E., Trapido-Rosenthal, H. G., and Douglas, A. E.: Functional significance of genetically different symbiotic algae *Symbiodinium* in a coral reef symbiosis, *Mol. Ecol.*, 16, 4849–4857, doi:10.1111/j.1365-294X.2007.03491.x, 2007.
- Marubini, F. and Thake, B.: Bicarbonate addition promotes coral growth, *Limnol. Oceanogr.*, 44, 716–720, 1999.
- Marubini, F., Ferrier-Pagès, C., Furla, P., and Allemand, D.: Coral calcification responds to seawater acidification: a working hypothesis towards a physiological mechanism, *Coral Reefs*, 27, 491–499, doi:10.1007/s00338-008-0375-6, 2008.
- Muscatine, L.: Glycerol excretion by symbiotic algae from corals and *Tridacna* and its control by the host, *Science*, 156, 516–519,

- 1967.
- Muscatine, L., McCloskey, L. R., and Marian, R. E.: Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration, *Limnol. Oceanogr.*, 26, 601–611, 1981.
- Muscatine, L., Falkowski, P. G., Porter, J. W., and Dubinsky, Z.: Fate of photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*, *Proc. R. Soc. Lond. B*, 222, 181–202, 1984.
- Reynaud, S., Leclercq, N., Romaine-Lioud, S., Ferrier-Pagès, C., Jaubert, J., and Gattuso, J. P.: Interacting effects of CO₂ partial pressure and temperature on photosynthesis and calcification in a scleractinian coral, *Glob. Change Biol.*, 9, 1660–1668, doi:10.1046/j.1365-2486.2003.00678.x, 2003.
- Rodolfo-Metalpa, R., Richard, C., Allemand, D., Bianchi, C. N., Morri, C., and Ferrier-Pagès, C.: Response of zooxanthellae in symbiosis with the Mediterranean corals *Cladocora caespitosa* and *Oculina patagonica* to elevated temperatures, *Mar. Biol.*, 150, 45–55, doi:10.1007/s00227-006-0329-x, 2006.
- Rodolfo-Metalpa, R., Martin, S., Ferrier-Pagès, C., and Gattuso, J.-P.: Response of the temperate coral *Cladocora caespitosa* to mid- and long-term exposure to pCO₂ and temperature levels projected for the year 2100AD, *Biogeosciences*, 7, 289–300, doi:10.5194/bg-7-289-2010, 2010.
- Santos, S. R., J., T. D., Kinzie, R. A., Hidaka, M., Sakai, K., and Coffroth, M. A.: Molecular phylogeny of symbiotic dinoflagellates inferred from partial chloroplast large subunit (23S)-rDNA sequences, *Mol. Phylogenet. Evol.*, 23, 97–111, 2002.
- Scheinder, K. and Erez, J.: The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*, *Limnol. Oceanogr.*, 51, 1284–1293, doi:10.4319/lo.2006.51.3.1284, 2006.
- Smith, P. K., Khrohn, R. I., Hermanson, G. T., Malia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C.: Measurement of protein using bicinchoninic acid, *Anal. Biochem.*, 150, 76–85, 1985.
- Tortell, P. D.: Evolutionary and ecological perspectives on carbon acquisition in phytoplankton, *Limnol. Oceanogr.*, 45, 744–750, 2000.
- Tremblay, P., Ferrier-Pagès, C., Maguer, J. F., Rottier, C., Legendre, L., and Grover, R.: Controlling effects of irradiance and heterotrophy on carbon translocation in the temperate coral *Cladocora caespitosa*, *PLoS ONE*, 7, e44672, doi:10.1371/journal.pone.0044672, 2012a.
- Tremblay, P., Grover, R., Maguer, J. F., Legendre, L., and Ferrier-Pagès, C.: Autotrophic carbon budget in the coral tissue: a new ¹³C-based model of photosynthate translocation, *J. Exp. Biol.*, 215, 1384–1393, doi:10.1242/jeb.065201, 2012b.
- Tremblay, P., Naumann, M. S., Sikorski, S., Grover, R., and Ferrier-Pagès, C.: Experimental assessment of organic carbon fluxes in the scleractinian coral *Stylophora pistillata* during a thermal and photo stress event, *Mar. Ecol. Prog. Ser.*, 453, 63–77, doi:10.3354/meps09640, 2012c.
- Veal, C. J., Carmi, M., Fine, M., and Hoegh-Guldberg, O.: Increasing the accuracy of surface area estimation using single wax dipping of coral fragments, *Coral Reefs*, 29, 893–897, doi:10.1007/s00338-010-0647-9, 2010.
- Venn, A., Tambutté, É., Holcomb, M., Laurent, J., Allemand, D., and Tambutté, S.: Impact of seawater acidification on pH at the tissue-skeleton interface and calcification in reef corals, *Proc. Natl. Acad. Sci. USA*, 110, 1634–1639, doi:10.1073/pnas.1216153110, 2013.